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CERTIFICATE

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I hereby certify that annexed is a true copy of the Provisional Specification as filed on 21 August 2002 with an application for Letters Patent number 520919 made by INDUSTRIAL RESEARCH LIMITED and ALBERT EINSTEIN COLLEGE OF MEDICINE OF YESHIVA UNIVERSITY.

Dated 10 September 2003.

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PROVISIONAL SPECIFICATION

INHIBITORS OF NUCLEOSIDE PHOSPHORYLASES AND NUCLEOSIDASES

We, **INDUSTRIAL RESEARCH LIMITED**, a New Zealand company of Brooke House, 24 Balfour Road, Parnell, Auckland, New Zealand and **ALBERT EINSTEIN COLLEGE OF MEDICINE OF YESHIVA UNIVERSITY**, a New York company of 1300 Morris Park Avenue, Bronx, New York 10461, United States of America, do hereby declare this invention to be described in the following statement:

INHIBITORS OF NUCLEOSIDE PHOSPHORYLASES AND NUCLEOSIDASES

TECHNICAL FIELD

This invention relates to certain nucleoside analogues, the use of these compounds as pharmaceuticals, pharmaceutical compositions containing the compounds, processes for preparing the compounds, and intermediates useful in the preparation of the compounds.

BACKGROUND OF THE INVENTION

US 5,985,848, US 6,066,722 and US 6,228,741 are directed to nucleoside analogues that are inhibitors of purine nucleoside phosphorylase (PNP) and purine phosphoribosyltransferases (PPRT). The analogues are useful in treating parasitic infections, T-cell malignancies, autoimmune diseases and inflammatory disorders. The analogues are also useful for immunosupression in organ transplantation. PCT/NZ00/00048 provides a process for preparing certain PNP inhibitor compounds. This application recognises the compounds as PNP inhibitors and addresses a need for simpler methods of preparing them. PCT/NZ01/00174 also provides further nucleoside analogues that are inhibitors of PNP and PPRT.

Certain nucleoside analogues have also been identified as potent inhibitors of 5'-methylthioadenosine phosphorylase (MTAP) and 5'-methylthioadenosine nucleosidase (MTAN). These are the subject of New Zealand patent application no. 517970.

PNP catalyses the phosphorolytic cleavage of ribo- and deoxyribonucleosides, for example those of guanine and hypoxanthine, to give the corresponding sugar-1-phosphate and guanine, hypoxanthine or other purine bases.

Humans deficient in purine nucleoside phosphorylase (PNP) suffer a specific T-cell immunodeficiency due to an accumulation of dGTP which prevents proliferation of stimulated T lymphocytes. Inhibitors against PNP are therefore immunosuppressive, and are active against T-cell malignancies and T-cell proliferative disorders.

Nucleoside hydrolases catalyse the hydrolysis of nucleosides. These enzymes are not found in mammals but are required for nucleoside salvage in some protozoan parasites. Some protozoan parasites use nucleoside phosphorylases either instead of or in addition to nucleoside hydrolases for this purpose. Inhibitors of nucleoside hydrolases and phosphorylases can be expected to interfere with the metabolism of the parasite and therefore be usefully employed against protozoan parasites.

MTAP and MTAN function in the polyamine biosynthesis pathway, in purine salvage in mammals, and in the quorum sensing pathways in bacteria. They respectively catalyse the reversible phosphorolysis of 5'-methylthioadenosine (MTA) to adenine and 5-methylthio-α-D-ribose-1-phosphate (MTR-1P), and the hydrolysis of MTA to adenine and 5-methylthio-α-D-ribose. The adenine formed is subsequently recycled and converted into nucleotides. Essentially, the only source of free adenine in the human cell is a result of the action of these enzymes. The MTR-1P is subsequently converted into methionine by successive enzymatic actions.

MTA is a by-product of the reaction involving the transfer of an aminopropyl group from decarboxylated S-adenosyl methionine to putrescine during the formation of spermidine. The reaction is catalyzed by spermidine synthase. The spermidine synthase is very sensitive to product inhibition by accumulation of MTA. Therefore, inhibition of MTAP or MTAN severely limits the polyamine biosynthesis and the salvage pathway for adenine in the cells. Likewise, MTA is the by-product of the bacterial synthesis of acylated homoserine lactones from S-adenosylmethionine (SAM) and acyl-acyl carrier proteins in which the subsequent lactonization causes release of MTA and the acylated homoserine lactone. The acylated homoserine lactone is a bacterial quorum sensing molecule in bacteria

that is involved in bacterial virulence against human tissues. Inhibition of MTAN or MTAP in microbes will prevent MTA removal, and subject the pathway to product inhibition, thereby decreasing production of the quorum sensing pathway and decreasing the virulence of microbial infections.

MTAP deficiency due to a genetic deletion has been reported with many malignancies. The loss of MTAP enzyme function in these cells is known to be due to homozygous deletions on chromosome 9 of the closely linked MTAP and *p16/MTS1* tumour suppressor gene. As absence of *p16/MTS1* is probably responsible for the tumour, the lack of MTAP activity is a consequence of the genetic deletion and is not causative for the cancer. However, the absence of MTAP alters the purine metabolism in these cells so that they are mainly dependent on the *de novo* pathway for their supply of purines. That makes these cells unusually sensitive to inhibitors like methotrexate and azaserine, that block the *de novo* pathway. Therefore, a combination therapy of methotrexate or azaserine with an MTAP inhibitor will have unusually effective anti-tumour properties.

MTAP inhibitors would also be very effective against parasitic infection such as malaria that infects red blood cells (RBCs), as they lack the *de novo* pathway for purine biosynthesis. Protozoan parasites depend entirely upon the purines produced by the salvage pathway for their growth and propagation. MTAP inhibitors will therefore kill these parasites without having any negative effect on the host RBCs, as RBCs are terminally differentiated cells and they do not synthesize purines, produce polyamines or multiply.

The imino sugar part of the compounds described in the patent specifications referred to above has the nitrogen atom located between C-1 and C-4 so as to form 1,4-dideoxy-1,4-imino-D-ribitol compounds. The location of the nitrogen atom in the ribitol ring may be critical for binding to enzymes. In addition, the location of the link between the sugar part and the nucleoside base analogue may be critical for enzyme inhibitory activity. The known compounds have that link at C-1 of the sugar ring.

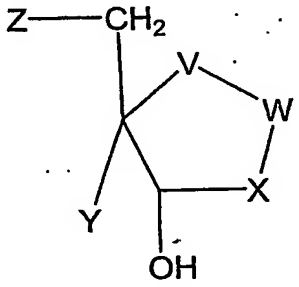
In the search for new and improved nucleoside phosphorylase and nucleosidase inhibitors, the applicants have investigated the synthesis and bioactivity of compounds where the location of the nitrogen atom in the sugar ring is varied and, additionally, where two nitrogen atoms form part of the sugar ring. Alternative modes of linking the sugar part and the base analogue have also been investigated.

The applicants have surprisingly found that certain novel compounds exhibit potent inhibitory activity against one or more of PNP, PPRT, MTAP and MTAN.

It is therefore an object of the present invention to provide compounds that are inhibitors of PNP, PPRT, MTAP and/or MTAN, or to at least provide the public with a useful choice.

SUMMARY OF THE INVENTION

In a first aspect of the invention there is provided a compound of the formula (I):



(I)

wherein:

V is selected from CH_2 and NH, and W is selected from NR^1 and NR^2; or

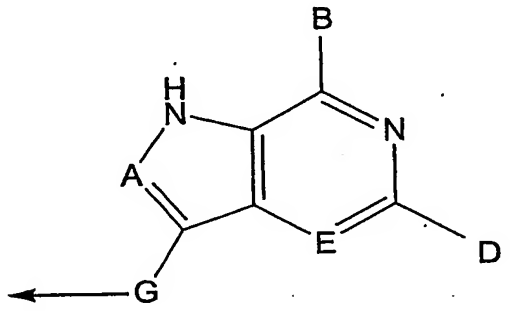
V is selected from NR^1 and NR^2, and W is selected CH_2 and NH;

X is selected from CH_2 and CHOH in the R or S-configuration, except where W is selected from NH, NR^1 and NR^2 then X is CH_2;

Y is selected from hydrogen, halogen and hydroxy, except where V is selected from NH, NR^1 and NR^2 then Y is hydrogen

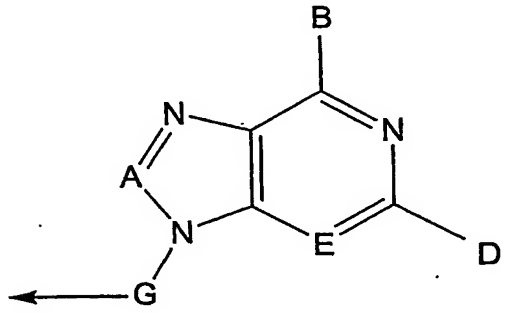
Z is selected from hydrogen, halogen, hydroxy, SQ and OQ, where Q is an optionally substituted alkyl, aralkyl or aryl group;

R^1 is a radical of the formula (II)



(II)

R^2 is a radical of the formula (III)



(III)

A is selected from N, CH and CR, where R is selected from halogen, optionally substituted alkyl, aralkyl or aryl, OH, NH_2, NHR^3, NR^3R^4 and SR^5, where R^3, R^4 and R^5 are each optionally substituted alkyl, aralkyl or aryl groups;

B is selected from OH, NH_2, NHR^6, SH, hydrogen and halogen, where R^6 is an optionally substituted alkyl, aralkyl or aryl group;

D is selected from OH, NH_2, NHR^7, hydrogen, halogen and SCH_3, where R^7 is an optionally substituted alkyl, aralkyl or aryl group;

E is selected from N and CH;

G is selected from CH_2 and NH, or G is absent, provided that where W is NR^1 or NR^2 and G is NH then V is CH_2, and provided that where V is NR^1 or NR^2 and G is NH then W is CH_2;

or a tautomer thereof, or a pharmaceutically acceptable salt thereof, or an ester thereof, or a prodrug thereof.

It is preferred that the compound of formula (I) is one where V is CH_2. It is further preferred that X is also CH_2.

Preferably W is NR^1. More preferably R^1 is a radical of formula (II) where A is CH, B is OH, D is hydrogen, E is N, and G is CH_2.

It is also preferred that when W is NR^1, A and E are both N, B is NH_2, D is hydrogen, and G is CH_2.

It is further preferred that V is NH and W is NR^1 or NR^2.

It will be appreciated that the representation of a compound of formula (I), where B and/or D is a hydroxy group, is of the enol-type tautomeric form of a corresponding amide, and this will largely exist in the amide form. The use of the enol-type tautomeric representation is simply to allow fewer structural formulae to represent the compounds of the invention.

Similarly, it will be appreciated that the representation of a compound of formula (I), where B and/or D is a thiol group, is of the thioenol-type tautomeric form of a corresponding thioamide, and this will largely exist in the thioamide form. The use of the thioenol-type tautomeric representation is simply to allow fewer structural formulae to represent the compounds of the invention.

In a second aspect of the invention there is provided a pharmaceutical composition comprising a pharmaceutically effective amount of a compound of formula (I).

In another aspect of the invention there is provided a method of treatment of diseases or conditions in which it is desirable to inhibit PNP, PPRT, MTAP, and/or MTAN. The method comprises administering a pharmaceutically effective amount of a compound of formula (I) to a patient requiring treatment. The diseases or conditions include cancer, bacterial and protozoal infections, and T-cell mediated diseases such as psoriasis, arthritis and transplant rejection.

In a further aspect of the invention there is provided the use of a compound of formula (I) in the manufacture of a medicament for the treatment of one or more of these diseases or conditions.

In still a further aspect of the invention there is provided a method of preparing a compound of formula (I).

In still a further aspect of the invention there is provided an intermediate useful in the preparation of a compound of formula (I).

DETAILED DESCRIPTION OF THE INVENTION

The compounds of the invention may be prepared by any method. However, preferably they are prepared by independently synthesising the sugar part and the

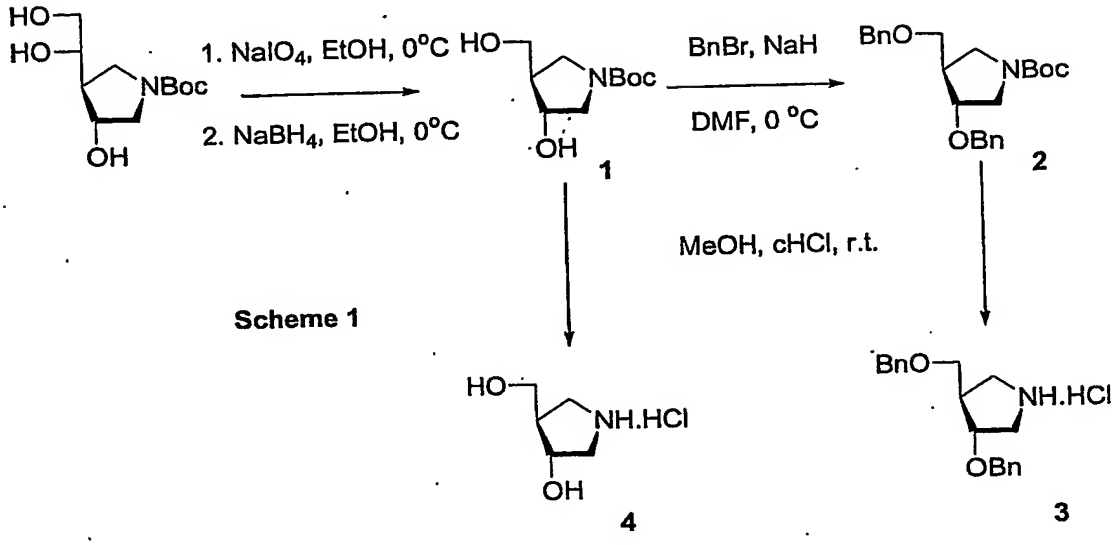
base part and then linking the base part to a nitrogen atom in the ring of the sugar part.

For example, Scheme 1 below outlines the preparation of the 1-*N*-imino sugar part of a compound of the invention where the nitrogen atom of the sugar analogue is located at the same position as the C-1 anomeric carbon atom would be found in a sugar molecule. A useful starting compound in the synthesis of the 1-*N*-iminosugar is N-*tert*-butoxycarbonyl-(3R,4S)-3-hydroxy-4-[(1S)-1,2-dihydroxyethyl] pyrrolidine. This starting compound may be prepared via the method of Filichev *et. al* [Carbohydrate Res., 2001, 333, 115-122] with the only variation being that a *t*-butoxycarbonyl moiety was utilised as the nitrogen protecting group rather than the N-(9-fluorenylmethoxycarbonyl) group. Oxidative cleavage of the diol moiety followed by reduction *in situ* gives the N-protected 3-hydroxy-4-hydroxymethylpyrrolidine (1). Removal of the N protecting group gives (3R,4R)-3-hydroxy-4-hydroxymethylpyrrolidine (4). Racemic 3-hydroxy-4-hydroxymethyl pyrrolidine was first prepared by Jaeger *et. al* [J. Org. Chem., 1965, 30, 740-744] and was used in the preparation of 1´-aza carbacyclic thymidine analogues [Lee, Y.H., Kim, H.K., Youn, I.K., Chae, Y.B., Bioorg. Med. Chem. Lett. 1991, 1, 287-290.] and aza-*C*-pyrimidines [Sorenson, M.D., Khalifa, N.M., Pedersen, E.B., Synthesis, 1999, 1937-1943].

Two other methods for the synthesis of (3R,4R)-3-hydroxy-4-hydroxymethylpyrrolidine have also been described. One method by Bols *et. al* [Bols, M., Hansen, S.U., Acta Chem. Scand., 1998, 52, 1214-1222] involves enzymatic purification of the enantiomers. The other method by Ichikawa *et. al* [Ichikawa, Y., Makino, K., Tetrahedron Lett., 1998, 39, 8245-8248] is a multi-gram asymmetric synthesis of (3R,4R)-3-hydroxy-4-hydroxymethylpyrrolidine via fumaric acid monoethyl ester. Ichikawa *et. al* evaluated the inhibitory activity of (3R,4R)-3-hydroxy-4-hydroxymethylpyrrolidine against human PNP and obtained an IC_{50} of 160μM.

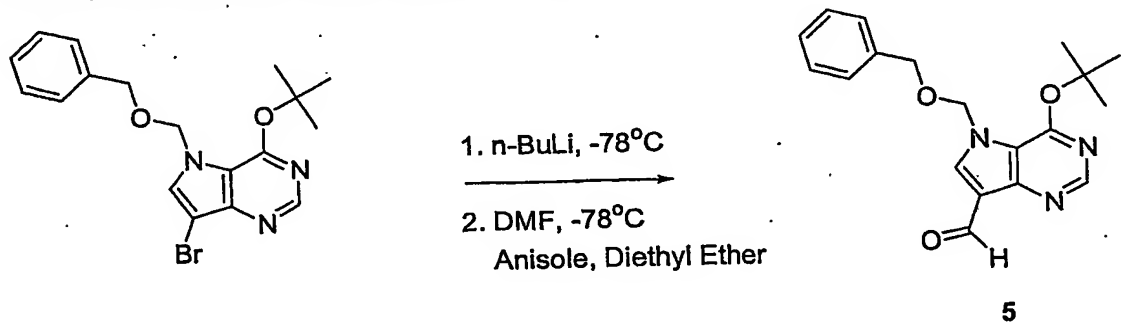
Benzylation of the hydroxyl groups of compound (1) before removal of the N protecting group may be desirable to give (3R,4R)-3-benzyloxy-4-

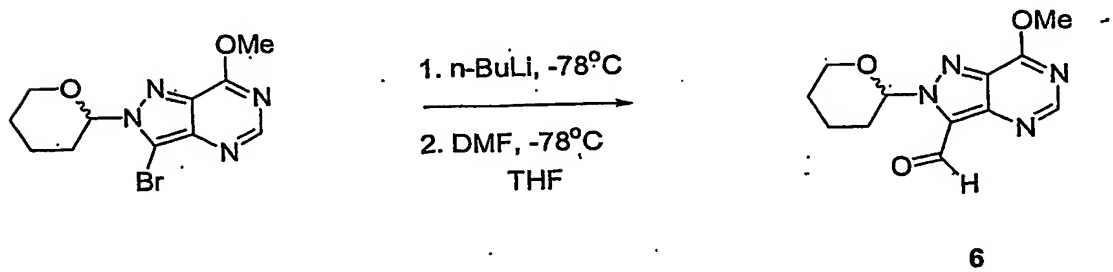
benzyloxymethylpyrrolidine hydrochloride (3) as a useful compound ready for linking to a suitable base analogue.



Scheme 1

The linking of the sugar part may be achieved by reductive amination of an appropriate aldehyde. Examples of suitable aldehydes, prepared from their corresponding bromo precursors, are shown in Scheme 2.

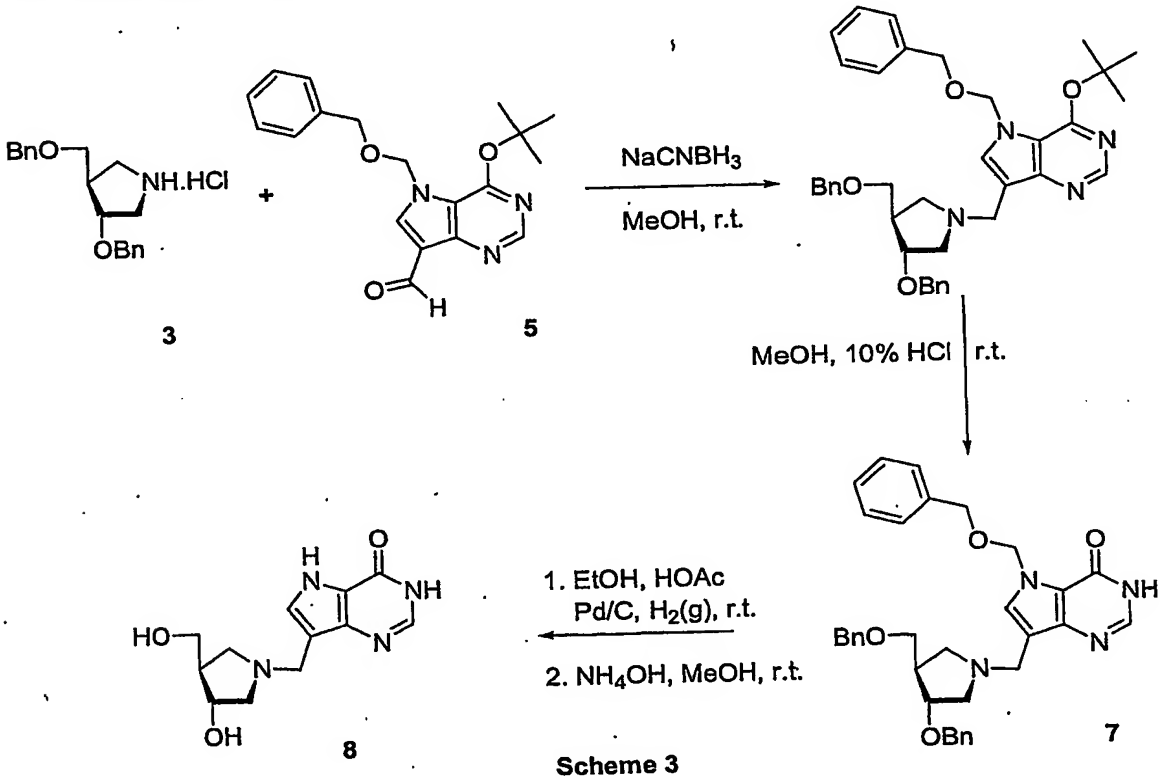




Scheme 2

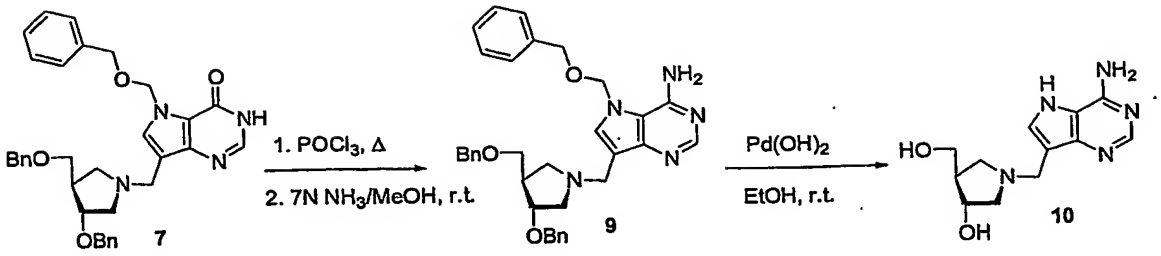
Coupling of an aldehydic base analogue with the protected sugar analogue (3) is shown in Scheme 3. Protecting group removal provides the desired inhibitor compound of the invention 7-[(3R,4R)-(3-hydroxy-4-hydroxymethylpyrrolidin-1-yl)methyl]-3H,5H-pyrrolo[3,2-d]pyrimidin-4-one (8).

It is to be appreciated that any sugar analogue having a nitrogen atom at any location in its ring may be coupled to any base analogue in this way. It is also to be appreciated that methods other than coupling by reductive amination of an aldehyde may be used.



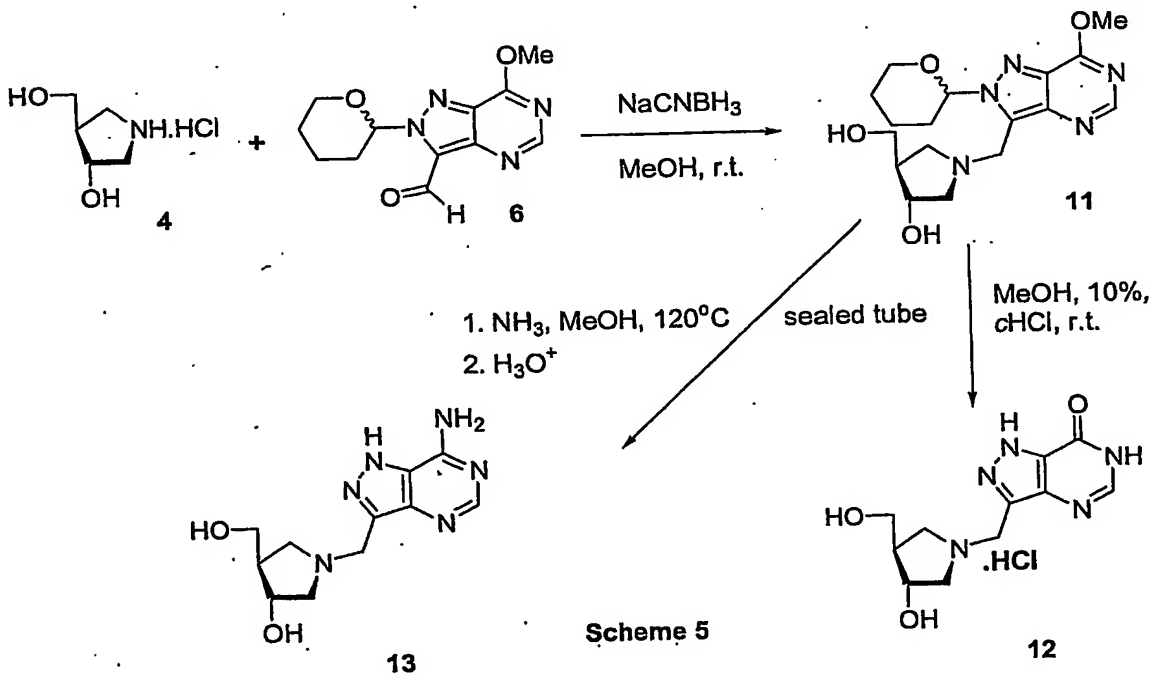
Scheme 3

As can be seen from Scheme 4 below, the intermediate (7) may be manipulated to afford (10).



Scheme 4

Other examples of the coupling of base analogues to the sugar analogue (4) are shown in Scheme 5. This method can be used to prepare the potential inhibitors 3-[(3R,4R)-3-hydroxy-4-hydroxymethylpyrrolidin-1-yl)methyl]-1H,6H-pyrazolo[4,3-d]pyrimidin-7-one (12) and 3-[(3R,4R)-3-hydroxy-4-hydroxymethylpyrrolidin-1-yl)methyl]-1H,6H-pyrazolo[4,3-d]-7-aminopyrimidine (13).

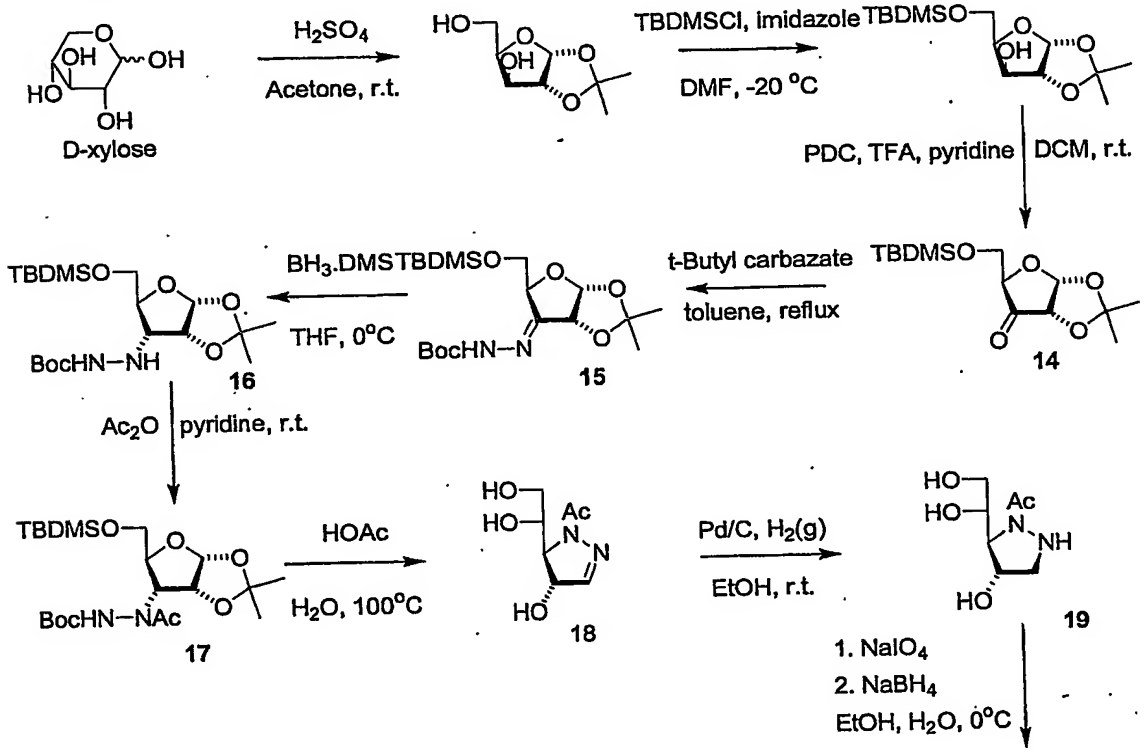


Scheme 5

An intermediate sugar analogue containing two nitrogen atoms in its ring has been prepared. (3R,4S)-4-Hydroxy-3-hydroxymethylpyrazolidine (21) may be prepared according to the route outlined in Scheme 6. The ketone (14) is prepared from D-xylose using well known chemistry (Lin, T-S., Zhu, J-L., Dutschman, G.E., Cheng,

Y-C., Prusoff, W.H., *J. Med. Chem.* 1993, 36, 353-362). Amination followed by reduction of the imine and acetylation of the resulting secondary amine gives compound (17). The key step of acid hydrolysis with concomitant recyclisation gives the imino cycle (18). Hydrogenation followed by cleavage of the diol moiety and removal of the acetate provides the desired pyrazolidine (21).

The pyrazolidine (21), or the precursor N-acetate (20), may be coupled with a variety of base analogues to give potential inhibitors of the formula (I) of this invention.



Scheme 6

The compounds of the invention are useful in both free base form and in the form of salts. The term "pharmaceutically acceptable salts" is intended to apply to non-toxic salts derived from inorganic or organic acids, including, for example, the

following acids: hydrochloric, sulphuric, phosphoric, acetic, lactic, fumaric, succinic, tartaric, gluconic, citric, methanesulfonic and *p*-toluenesulfonic acids.

The active compounds may be administered to a patient by a variety of routes, including oral administration, injection, or topical administration. The amount of compound to be administered will vary widely according to the nature of the patient and the nature and extent of the disorder to be treated. Typically the dosage for an adult human will be in the range less than 1 to 1000 milligrams, preferably 0.1 to 100 milligrams.

For oral administration the compounds can be formulated into solid or liquid preparations, for example tablets, capsules, powders, solutions, suspensions and dispersions. Such preparations are well known in the art as are other oral dosage regimes not listed here. In the tablet form the compounds may be tableted with conventional tablet bases such as lactose, sucrose and corn starch, together with a binder, a disintegration agent and a lubricant. The binder may be, for example, corn starch or gelatin, the disintegrating agent may be potato starch or alginic acid and the lubricant may be magnesium stearate. Other components such as colourings or flavourings may be added.

Liquid forms include carriers such as water and ethanol, with or without other agents such as a pharmaceutically acceptable surfactant or suspending agent.

The compounds may also be administered by injection in a physiologically acceptable diluent such as water or saline. The diluent may comprise one or more other ingredients such as ethanol, propylene glycol, an oil or a pharmaceutically acceptable surfactant.

The compounds may be present as ingredients in creams, for topical administration to skin or mucous membranes. Preferably the creams include a pharmaceutically acceptable solvent to assist passage through the skin or mucous membranes. Suitable creams are well known to those skilled in the art.

The compounds may further be administered by means of sustained release systems. For example, they may be incorporated into a slowly dissolving tablet or capsule.

EXAMPLES

The following examples further illustrate the invention. It is to be appreciated that the invention is not limited to the examples.

Example 1

N-*tert*-Butoxycarbonyl-(3R,4R)-3-hydroxy-4-hydroxymethylpyrrolidine (1). N-*tert*-Butoxycarbonyl-(3R,4S)-3-hydroxy-4-[(1S)-1,2-dihydroxyethyl]pyrrolidine (3.4 g, 13.7 mmol) in ethanol (50 mL) was added dropwise to a stirred solution of sodium periodate (3.4 g, 16 mmol) in water (25 mL) while maintaining the reaction temperature at 0 °C. The reaction was left an additional 20 min after which time sodium borohydride (2.0 g, excess) was added portionwise while again ensuring the reaction temperature was maintained at 0 °C. On complete addition the solid was filtered, washed with ethanol (50 mL) and concentrated in vacuo to afford a syrup. Chromatography afforded **1** (2.74 g, 92%) as a syrup.

Example 2

N-*tert*-Butoxycarbonyl-(3R,4R)-3-benzyloxy-4-benzyloxymethylpyrrolidine (2). Sodium hydride (140 mg, 60% oil dispersion, 3.7 mmol) was added portionwise to a stirred solution of benzyl bromide (300 L, 2.8 mmol) and **1** (200 mg, 0.92 mmol) in DMF (10 mL) at 0°C. On complete addition the resulting suspension was allowed to warm to r.t., diluted with toluene (100 mL), washed with water (50 mL), brine (50 mL), dried ($MgSO_4$), and concentrated in vacuo to afford a syrup. Chromatography afforded **2** (350 mg, 96%) as an oil which was used in the next step without purification.

Example 3

(3R,4R)-3-Benzyloxy-4-benzyloxymethylpyrrolidine hydrochloride (3). Hydrochloric acid (2 mL, 1M) was added to a solution of **2** (500 mg, 1.3 mmol) in methanol (2 mL) and the resulting mixture stirred for 1 h at 40°C. On completion the reaction was concentrated *in vacuo* to afford **3** as the hydrochloride salt (330 mg, 90%). ^{1}H NMR 7.35–7.21 (m,10H), 4.48 (m, 4H), 4.08 (d, J = 2.9 Hz,1H), 3.53 (m, 1H), 3.44 (m, 3H), 3.24 (m, 1H), 2.65 (m, 1H). ^{13}C NMR 138.0, 137.6, 128.9, 128.8, 128.3, 128.2, 79.3, 73.7, 71.9, 68.7, 49.6, 46.4, 44.8.

Example 4

(3R,4R)-3-Hydroxy-4-hydroxymethylpyrrolidine (4). Hydrochloric acid (5 mL, 12M) was added dropwise to a stirred solution of **1** (2.3 g, 10.6 mmol) in methanol (5 mL) at room temperature. After 1 h the reaction was concentrated in vacuo to afford **4** (1.63 g, 100%) as an oil. ^{13}C NMR 71.9, 60.9, 52,1, 47.9, 46.6.

Example 5

5-N-Benzyloxymethyl-4-*tert*-butoxypyrrolo[3,2-d]pyrimidine-7-carbaldehyde (5). 5-Benzyloxymethyl-7-bromo-4-*tert*-butoxypyrrolo[3,2-d]pyrimidine (400 mg, 1.02 mmol) was dissolved in diethyl ether (10 mL) and anisole (5 mL) and cooled to –78 °C. *n*-Butyl lithium (600 L, 2.5 M) was then added dropwise at such a rate as to maintain the reaction temperature below –70 °C and the resulting solution left for 30 min at –78 °C. Dimethylformamide (100 L) was then added and the reaction left stirring for an additional 30 min and then quenched with water and allowed to warm to r.t.. The reaction was then diluted with ethyl acetate (100 mL), washed with water (30 mL), brine (30 mL), dried (MgSO4) and concentrated *in vacuo* to afford a syrup. Purification by chromatography afforded **5** (270 mg, 78%). ^{1}H NMR 10.29 (s, 1H), 8.62 (s, 1H), 7.98 (s, 1H), 7.34 – 7.22 (m, 5H), 5.79 (s, 2H), 4.53 (s, 2H), 1.71 (s, 9H). ^{13}C NMR 184.8, 156.63, 152.6, 150.0, 136.7, 136.6, 128.9, 128.5, 127.8, 118.4, 84.4, 78.3, 71.0, 29.0.

Example 6

7-Methoxy-2-N-(tetrahydropyran-2-yl)pyrazolo[4,3-d]pyrimidine-3-carbaldehyde (6). *n*-BuLi (0.7 mL, 2.4 M) was added dropwise to a stirred solution of 3-Bromo-7-

methoxy-2-N-(tetrahydropyran-2-yl)pyrazolo[4,3-d]pyrimidine (530 mg, 1.7 mmol) in THF (20 mL) at −78 °C under an inert atmosphere. The reaction was stirred for an additional 30 min at −78 °C and then DMF (1.0 mL) was added and the reaction allowed to warm to room temperature. The reaction was quenched with water (50 mL) extracted with toluene (2 x 100 mL), the organic layers were combined washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo to afford a solid residue. Chromatography afforded **6** as a solid. ^{1}H NMR 10.43 (s, 1H), 8.71 (s, 1H), 6.55 (dd, J = 10.0, 2.7 Hz, 1H), 4.25 (s, 3H), 4.13 (m, 1H), 3.83 (dt, J = 10.8, 2.8 Hz), 2.53 – 1.65 (m, 7H). ^{13}C NMR 177.0, 161.5, 154.5, 143.9, 130.2, 128.9, 87.0, 67.4, 53.5, 28.7, 23.7, 21.2.

Example 7

7-[(3R,4R)-(3-Benzyloxy-4-benzyloxymethylpyrrolidin-1-yl)methyl]-5-benzyloxymethyl-3H-pyrrolo[3,2-d]pyrimidin-4-one (7). Sodium cyanoborohydride (100 mg, 1.59 mmol) was added to a stirred solution of **5** (220 mg, 0.64 mmol) and **3.HCl** (190 mg, 0.57 mmol) in methanol (5 mL) and stirred overnight at r.t.. The reaction was then concentrated *in vacuo* and redissolved in methanol (2 mL) and cHCl (2 mL), stirred for 1 h and then concentrated in vacuo to afford a solid residue. Chromatography of the resulting residue afforded **7** (202 mg, 63%) as a solid. ^{1}H NMR 7.87 (1H, s), 7.32 (1H, s), 7.31-7.23 (m, 5H), 5.89 (s, 2H), 4.56 (s, 2H), 4.50 (s, 2H), 4.48 (s, 2H), 4.47 (s, 2H), 3.87 (m, 2H), 3.81 (q, J = 13.4 Hz, 2H), 3.43 (d, J = 7.1 Hz, 2H), 3.01 (t, J = 8.1 Hz, 1H), 2.79 (d, J = 4.7 Hz, 1H), 2.55 (m, 1H), 2.36 (m, 1H). ^{13}C NMR 156.2, 145.8, 141.8, 138.9, 138.8, 137.6, 131.4, 128.8, 128.7, 128.7, 128.3, 128.2, 128.1, 128.0, 128.8, 117.9, 115.7, 81.3, 77.1, 73.5, 72.1, 71.4, 70.8, 60.0, 56.4, 48.6, 45.9.

Example 8

7-[(3R,4R)-(3-Hydroxy-4-hydroxymethylpyrrolidin-1-yl)methyl]-3H,5H- pyrrolo[3,2-d]pyrimidin-4-one (8). Compound **7** (120 mg, 0.21 mmol) and Pearlmans catalyst (120 mg) were suspended in ethanol (3 mL) and acetic acid (1 mL) and vigorously stirred under an atmosphere of hydrogen gas for 24 h at r.t.. The reaction was then filtered through celite and concentrated *in vacuo* to afford a solid.

Chromatography and ion exchange of the solid afforded **8** (38 mg, 68%) as a white solid with m.p. 248–250 °C. ^{1}H NMR 7.81 (1H, s), 7.34 (1H, s), 3.97 (1H, brs), 3.65 (2H, s), 3.53 (1H, m), 3.44 (1H, m), 2.93 (1H, t, J = 9.0Hz), 2.77 (1H, m), 2.60 (1H, m), 2.33 (1H, t, J = 7.1Hz), 2.12 (1H, brs). ^{13}C NMR 155.8, 144.1, 142.8, 130.0, 117.3, 111.1, 72.9, 62.7, 60.2, 54.8, 48.9, 47.3. HRMS (MH+) calc. for $C_{12}H_{18}N_4O_3$: 265.1301. Found 265.1302. Anal. Calc. for $C_{12}H_{16}N_4O_3.\frac{1}{2}H_2O$ C, 52.7; H, 6.2; N, 20.5. Found C, 53.0; H 5.9; N, 20.4.

Example 9

7-[(3R,4R)-(3-Benzyloxy-4-benzyloxymethylpyrrolidin-1-yl)methyl]-4-amino-5-benzyloxymethylpyrrolo[3,2-d]pyrimidine (9). Compound **7** (1.2 g, 2.12 mmol) was added to phosphoryl chloride (20 mL) and the resulting suspension heated to reflux. After 1h the reaction was concentrated *in vacuo*, diluted with chloroform, washed with saturated $NaHCO_3$, brine, dried ($MgSO_4$), and concentrated *in vacuo*. The resulting residue was redissolved in 7N NH_3 in methanol and the resulting solution heated to 120 °C in a sealed tube overnight. The reaction was concentrated in vacuo and purified by chromatography to afford **9** (0.83 g, 69%). ^{1}H NMR 8.38 (s, 1H), 7.76 (brs, 1H), 7.32 – 7.25 (m, 15H), 6.01 (brs, 2H), 5.51 (d, J = 2.3 Hz, 2H), 4.55 (s, 2H), 4.51 (s, 2H), 4.48 (s, 2H), 4.25 (d, J = 2.9 Hz, 2H), 4.05 (m, 1H), 3.50 (d, J = 6.5 Hz, 2H), 3.42 (m, 1H), 3.31 (m, 1H), 3.20 (m, 1H), 3.01 (m, 1H), 2.71 (m, 1H). ^{13}C NMR 152.3, 151.5, 150.1, 138.3, 138.0, 135.7, 134.6, 129.2, 129.0, 128.8, 128.2, 128.1, 115.18, 107.94, 79.7, 77.6, 73.6, 71.9, 70.7, 69.8, 58.6, 58.1, 55.22, 54.9, 48.8, 45.3.

Example 10

(3R,4R)-3-Hydroxy-4-hydroxymethyl-1-[7-methoxy-2-(tetrahydropyran-2-yl)-2H-pyrazolo[4,3-d]pyrimidin-3-ylmethyl]-pyrrolidine (11). Sodium cyanoborohydride (100 mg, 1.59 mmol) was added to a stirred solution of **6** (340 mg, 1.3 mmol) and **4.HCl** (190 mg, 0.57 mmol) in methanol (5 mL) and stirred overnight at r.t.. Chromatography of the resulting residue afforded **11** (150 mg, 35%) as a solid. ^{1}H NMR 8.39 (s, 1H), 5.90 (d, *J* = 9.1 Hz, 1H), 4.17 – 3.94 (m, 4H), 4.12 (s,

3H), 3.67 – 3.52 (m, 2H), 2.94 – 2.79 (m, 2H), 2.66 – 2.52 (m, 2H), 2.35 – 2.09 (m, 2H), 1.70 – 1.56 (m, 2H). ^{13}C NMR 162.6, 152.2, (140.1, 140.0), 133.5, 131.6, (87.0, 86.9), 74.3, (68.3, 68.2), (64.3, 64.2), 62.6, (56.2, 56.1), 54.5, (50.6, 50.7), (47.7, 47.6), 29.7, 25.2, 21.8.

Example 11

3-[(3R,4R)-3-Hydroxy-4-hydroxymethylpyrrolidin-1-yl)methyl]-1H,6H -pyrazolo[4,3-d]pyrimidin-7-one (12). Concentrated hydrochloric acid (1 mL, 12M) was added to a solution of **11** (50 mg, 0.14 mmol) in methanol and stirred overnight and then concentrated *in vacuo* to afford a solid residue which was triturated with methanol and filtered to afford **12** (38 mg, 92%) as a solid. ^{1}H NMR 8.13 (s, 1H), 4.35 (d, J = 2.7 Hz, 1H), 3.86 (m, 1H), 3.66 – 3.43 (m, 2H), 3.55 (d, J = 5.7 Hz, 2H), 3.10 (m, 1H), 2.44 (brs, 1H). ^{13}C NMR 154.7, 145.4, 137.1, 134.7, 128.6, 71.4, 60.6, 60.6, 55.0, 48.0, 47.9.

Example 12

3-[(3R,4R)-3-Hydroxy-4-hydroxymethylpyrrolidin-1-yl)methyl]-1H,6H -pyrazolo[4,3-d]-7-aminopyrimidine (13). A solution of **11** (100 mg) in 7 N NH_3 in methanol (4mL) was heated in a sealed tube at 120 °C overnight. The reaction was then concentrated *in vacuo* and the crude residue redissolved in methanol (1 mL) and cHCl (1 mL) and allowed to stand overnight. The reaction was concentrated again *in vacuo* and the resulting residue purified by chromatography to afford **13** (61 mg, 84%). ^{13}C NMR 152.4, 151.5, 139.1, 134.8, 122.7, 71.7, 61.1, 60.3, 55.0, 48.4, 48.2.

Example 13

5-*O*-*tert*-Butyldimethylsilyl-1,2-*O*-isopropylidene- -D-*erythro*-pentofuranos-3-ulose (*tert*-butoxycarbonyl)hydrazone (15). A toluene (150 mL) solution of **14** (11.5 g, 38 mmol), tert-butyl carbazate (17 g, 128 mmol) and pyridinium *p*-toluenesulfonate (1.15 g, 4.6 mmol) was stirred overnight at 70 °C. On completion the reaction was washed with saturated $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated *in vacuo* to afford a syrup. Purification by chromatography afforded **15** (12.5 g, 79%) as an oil. ^{1}H NMR 8.43 (brs, 1H),

5.98 (d, J = 4.8 Hz, 1H), 4.90 (dd, J = 4.8, 1.5 Hz, 1H), 4.76 (q, J = 1.5 Hz, 1H), 3.77 (m, 2H), 1.48 (s, 9H), 1.45 (s, 3H), 1.41 (s, 3H), 0.82 (s, 9H), -0.03 (d, J = 5.8 Hz, 6H). ^{13}C NMR 153.5, 152.8, 114.3, 105.7, 82.0, 81.7, 76.2, 66.2, 28.6, 28.0, 27.5, 26.2, 18.5. HRMS (MH^+) calc. for $C_{19}H_{37}N_2O_6Si$: 417.2421. Found 417.2398.

Example 14

3-(2-*tert*-Butoxycarbonylhydrazino)-5-*O*-*tert*-butyldimethylsilyl-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (16). Borane.DMS complex (15 mL, ~10M, 150 mmol) was added dropwise to a stirred solution of **15** (12.5 g, 30 mmol) at −78 °C under an inert atmosphere. The reaction was allowed to warm to r.t., quenched cautiously with methanol and then the resulting solution concentrated *in vacuo*. The crude syrup obtained was co-distilled with aliquots of methanol (3 x 100 mL) to afford **16** (12.5 g, 100%) as an oil which was used in the next step without further purification. ^{1}H NMR 6.29 (brs, 1H), 5.67 (d, J = 3.7 Hz, 1H), 4.62 (t, J = 4.3 Hz, 1H), 4.24 (brs, 1H), 3.75 (m, 2H), 3.72 (m, 1H), 1.46 (s, 3H), 1.38 (s, 9H), 1.27 (s, 3H), 0.82 (s, 9H), -0.03 (s, 6H). ^{13}C NMR 156.9, 112.8, 104.6, 80.7, 80.4, 80.2, 65.0, 63.1, 28.7, 27.1, 26.9, 26.3, 18.7. HRMS (MH^+) calc. for $C_{19}H_{38}N_2O_6Si$: 418.2499. Found 418.2509.

Example 15

3-(1-Acetyl-2-*tert*-butoxycarbonylhydrazino-5-*O*-*tert*-butyldimethylsilyl-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (17). Acetic anhydride (10 mL, xs) was added to a stirred solution of **16** (12.5 g, 30 mmol) in pyridine (30 mL) and the resulting reaction allowed to stir overnight at r.t.. On completion the reaction was diluted with chloroform (500 mL) and washed with 10% HCl, water, saturated $NaHCO_3$, brine and then the organic layer was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo* to afford a crude yellow oil. Purification by chromatography afforded **17** (6.5 g, 47%) as a colourless oil. ^{1}H NMR 7.20 (brs, 1H), 5.75 (d, J = 3.8 Hz, 1H), 5.02 (dd, J = 9.8, 5.0 Hz, 1H), 4.72 (t, J = 4.2 Hz, 1H), 4.04 (dd, J = 9.8, 2.2 Hz, 1H), 3.87 (d, J = 11.6 Hz, 1H), 3.70 (dd, J = 11.6, 3.8 Hz, 1H), 2.09 (s, 3H),1.55 (s, 3H), 1.42 (s, 9H), 1.28 (s, 3H), 0.84 (s, 9H), -0.03 (s, 6H). ^{13}C NMR 174.5, 155.2, 112.7, 104.7, 82.0, 81.0, 77.2,

62.9, 62.0, 55.0, 28.5, 27.0, 26.7, 26.3, 21.1, 18.7. HRMS (MH^+) calc. for $C_{21}H_{41}N_2O_7Si$: 461.2683. Found 461.2704.

Example 16

(3S,4S)-2-Acetyl-3,4-dihydro-3-[(1S)-1,2-dihydroxyethyl]-4-hydroxypyrazole (18). A stirred solution of **17** (2.0 g, 4.3 mmol) in 70% acetic acid (20 mL) was heated at 100 °C overnight. The resulting solution was allowed to cool, diluted with water (100 mL) and the aqueous solution extracted with chloroform (2 x 100 mL) and then the aqueous layer was concentrated *in vacuo* to afford a syrup. The product was purified by chromatography to afford **18** (380 mg, 47%) as an oil. ^{13}C NMR 173.1, 150.5, 112.7, 74.7, 69.6, 65.4, 62.4, 21.4. HRMS (MH^+) calc. for $C_7H_{13}N_2O_4$: 189.0875. Found 189.0876.

Example 17

(3S,4S)-2-Acetyl-3-[(1S)-1,2-dihydroxyethyl]-4-hydroxypyrazolidine (19). Pearlmans catalyst (200 mg) was suspended in a solution of methanol with (3S,4S)-2-acetyl-1,5-dihydro-3-[(1S)-1,2-dihydroxyethyl]-4-hydroxy-pyrazole **(18)** (200 mg, 1.11 mmol) and stirred overnight under an atmosphere of hydrogen. The reaction was filtered through celite and the filtrated concentrated in vacuo to afford a crude oil. The crude product was purified by chromatography to afford **19** (85 mg, 43%) as a colourless oil. ^1H NMR 4.64 (dd, J = 5.7, 3.4 Hz, 1H), 3.90 (m, 2H), 3.61 (m, 2H), 3.36 (dd, J = 11.7, 6.5 Hz, 1H), 2.71 (dd, J = 11.7, 6.0 Hz, 1H), 2.18 (s, 3H). ^{13}C NMR 174.5, 75.4, 72.7, 68.2, 65.2, 56.4, 21.5. HRMS (M^+) calc. for $C_7H_{14}N_2O_4$: 190.0953. Found 190.0951.

Example 18

(3R,4S)-2-Acetyl-4-hydroxy-3-hydroxymethyl-pyrazolidine (20). A solution of **19** (80 mg, 0.42 mmol) in ethanol (5 mL) was added dropwise to a stirred solution of sodium periodate (150 mg, 0.7 mmol) in water (5 mL) at such a rate so as to maintain the reaction temperature at 5 °C. On completion, sodium borohydride (135 mg, xs) was added portionwise to the resulting suspension at such a rate so as to maintain the reaction temperature at 0 °C and on complete addition the reaction was allowed to warm to r.t.. Flash chromatography grade silica was

added to the reaction and the resulting suspension was concentrated *in vacuo* to afford a white solid. The solid was purified by chromatography to afford **20** (51 mg, 76%) as a colourless oil. ^{1}H NMR 4.43 (dd; J = 5.7, 3.3 Hz, 1H), 3.91 (q, J = 4.7 Hz, 1H), 3.75 (d, J = 4.7 Hz, 1H), 3.31 (m, 1H), 3.28 (dd, J = 11.8, 5.7 Hz, 1H), 2.75 (dd, J = 11.8, 5.5 Hz, 1H), 2.17 (s, 3H). ^{13}C NMR 173.9, 76.5, 68.2, 62.5, 55.8, 21.7. HRMS (MH^+) calc. for $C_6H_{13}N_2O_3$: 161.0926. Found 161.0920.

Example 19

(3R,4S)-4-Hydroxy-3-hydroxymethylpyrazolidine (21). Concentrated HCl (1.5 mL) was added dropwise to a stirred solution of **20** (1.5 mg, 0.09 mmol) in methanol (1.5 mL) and the resulting reaction kept at 60 °C for 3 h. The reaction was concentrated *in vacuo* to afford **21** (18 mg, 100%) as its dihydrochloride salt. ^{1}H NMR 4.60 (q, J = 2.4 Hz, 1H), 3.73 – 3.31 (m, 5H). ^{13}C NMR 72.6, 68.3, 60.2, 54.1.

Example 20

Inhibition of Purine Nucleoside Phosphorylase by Compound (8)

The reaction involves the conversion of inosine (1 mM) and inorganic phosphate (50 mM, pH 7.4) to hypoxanthine and α-D-ribose 1-phosphate. Analysis by this method requires that the inhibitor concentration be present at least 10x the enzyme concentration. Enzyme was present at 1.6 pM. The reaction progress was followed in a coupled assay by monitoring the formation of uric acid from oxidation of hypoxanthine by xanthine oxidase (128 g; 59 munits/ml reaction mixture). The inhibitor concentration from 0 to 1 nM was used to determine the initial dissociation constant. K_i was determined from the time interval of 0 to 4 min and the equilibrium dissociation constant K_i^* was determined from the time interval from 35 to 45 min. Inhibition constants (K_i or K_i^*) were determined according to the equations $v = (k_{cat})(A)/(K_m.(1 + I/K_i) + A)$ for K_i or $v = (k_{cat})(A)/(K_m.(1 + I/K_i^*) + A)$ for K_i^*. Slow-onset inhibition was apparent for the human enzyme with both Imm-H and compound (8), and thus yielded values of both K_i and K_i^*. Inhibition was immediate and a second phase of slow-onset

inhibition was not observed for the *P. falciparum* enzyme with ImmH or compound (8) or for the *M. tuberculosis* PNP with Imm-H. Thus, only the K_i values were observed.

The kinetic curves for human PNP, inhibited by compound (8) are shown in Figure 1. K_i* values for three PNP enzymes are shown in Table 1.

Figure 1. Slow-onset inhibition of human PNP by compound (8).

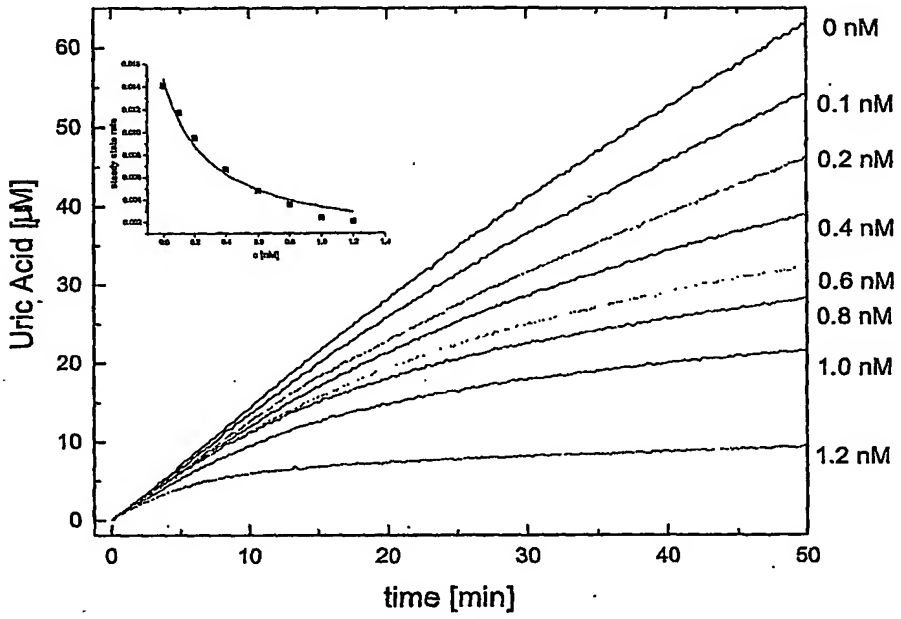
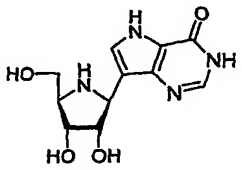
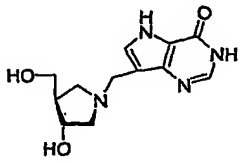


Table 1. Comparision of inhibition constants for ImmH and compound (8) for different PNP enzymes. Each value was obtained from 3-4 individual determinations of the kinetic constants.

	Human PNP	*P. falciparum* PNP	*M. tuberculosis* PNP
Imm-H 	$K_i = 3.3 \pm 0.2$ nM $K_i^* = 0.056 \pm 0.015$ nM	$K_i^* = 0.86 \pm 0.08$ nM	$K_i^* = 2.6 \pm 0.35$ nM
Compound (8) 	$K_i = 1.07 \pm 0.29$nM $K_i^* = 0.013 \pm 0.002$ nM	$K_i^* = 0.5 \pm 0.04$ nM	$K_i = 3.16 \pm 0.8$ nM $K_i^* = 0.082 \pm 0.010$ nM

Although the invention has been described by way of examples, it should be appreciated the variations or modifications may be made without departing from the scope of the invention. Furthermore, when known equivalents exist to specific features, such equivalents are incorporated as if specifically referred to in the specification.

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